APA143Hu01 10μg
Active Vascular Endothelial Growth Factor A (VEGFA)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Pro28~Arg147
Tags: N-terminal His-tag
Purity: >98%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 6.3
Predicted Molecular Mass: 18.2kDa
Accurate Molecular Mass: 17&18kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

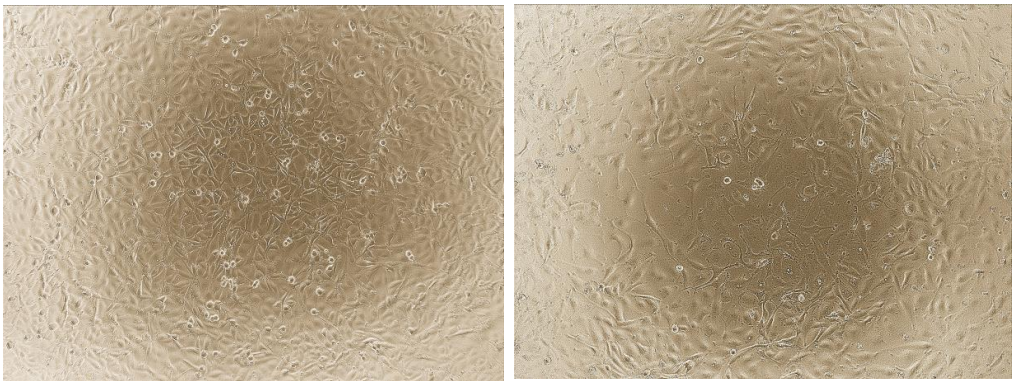
[SEQUENCE]

PMA EGGGQNHHEV VKFMDVYQRS YCHPIETLVD IFQEYPDEIE YIFKPSCVPL MRCGGCCNDE GLECVPTEES NITMQIMRIK PHQGQHIGEM SFLQHNKCEC RPKKDRARQE KCDKPRR

[ACTIVITY]

Vascular endothelial growth factor A (VEGFA) is a protein that in humans is encoded by the VEGFA gene. This protein is a glycosylated mitogen that specifically acts on endothelial cells and has various effects, including mediating increased vascular permeability, inducing angiogenesis, vasculogenesis and endothelial cell growth, promoting cell migration, and inhibiting apoptosis. VEGFA shows prominent activity with vascular endothelial cells, primarily through its interactions with the VEGFR1 and -R2 receptors found in prominently on the endothelial cell membrane. Thus, proliferation assay of recombinant human VEGFA was conducted using ECV-304 cells. Briefly, ECV-304 cells were seeded into triplicate wells of 96-well plates at a density of 2,000 cells/well and allowed to attach overnight, then the medium was replaced with serum-free standard

1640 prior to the addition of various concentrations of VEGFA. After incubated for 48h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10μL of CCK-8 solution was added to each well of the plate, then the absorbance at 450nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37℃. Proliferation of ECV-304 cells after incubation with VEGFA for 48h observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with human recombinant VEGFA for 48h. The result was shown in Figure 2. It was obvious that VEGFA significantly increased cell viability of ECV-304 cells.



A B

Figure 1. Cell proliferation of ECV-304 cells after stimulated with VEGFA.

(A) ECV-304 cells cultured in 1640, stimulated with 100ng/mL VEGFA for 48h;

(B) Unstimulated ECV-304 cells cultured in 1640 for 48h.

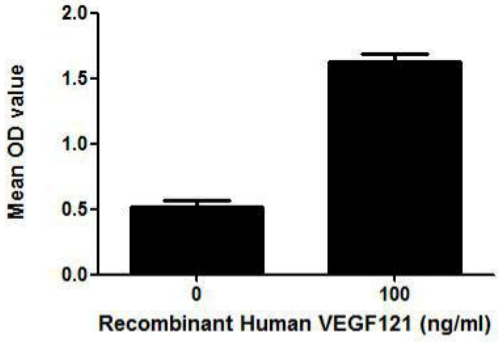


Figure 2. Cell proliferation of ECV-304 cells after stimulated with VEGFA.

[IDENTIFICATION]

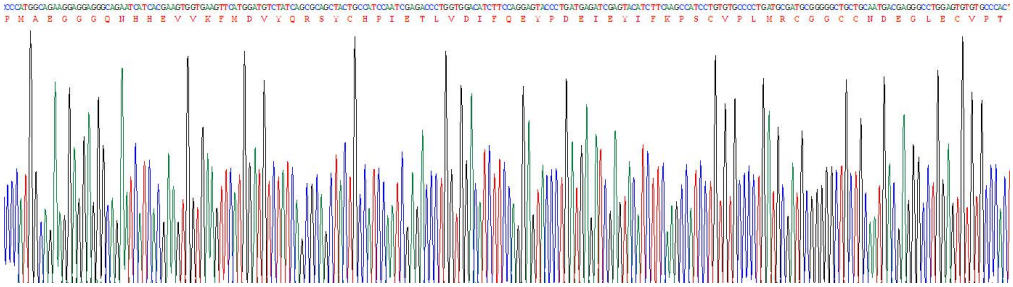


Figure 3. Gene Sequencing (extract)

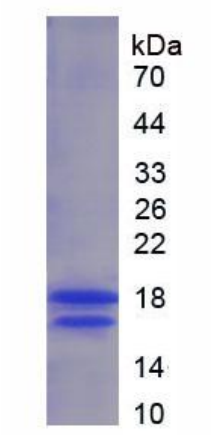


Figure 4. SDS-PAGE

Sample: Active recombinant VEGFA, Human

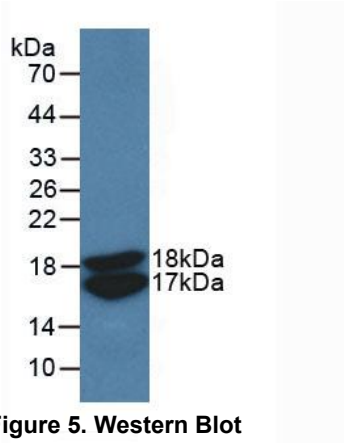


Figure 5. Western Blot

Sample: Recombinant VEGFA, Human;

Antibody: Rabbit Anti-Human VEGFA Ab (PAA143Hu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.